...erate stripping solution
200 mM Tris·Cl, pH 7.0
0.1× SSC (APPENDIX 2)
0.1% (w/v) SDS

Nucleotide mix
2.5 mM ATP
2.5 mM CTP
2.5 mM GTP
20 mM Tris·Cl, pH 7.5
Store at −20°C

...MMENTARY

...kground Information

...ybridization between complementary ...nucleotides was implicit in the Watson-... model for DNA structure and was ini-...ly exploited, via renaturation kinetics, as a ... of studying genome complexity. In these ... applications, the two hybridizing mole-... were both in solution—an approach that ...ll utilized in "modern" techniques such ...ase protection transcript mapping (UNITS ...) and oligonucleotide-directed mutagen-... (Chapter 8). The innovative idea of im-...lizing one hybridizing molecule on a solid ...rt was first proposed by Denhardt (1966) ...ed to methods for identification of specific ...nces in genomic DNA (dot blotting; UNIT ...nd recombinant clones (UNITS 6.3 & 6.4). A ... dimension was subsequently intro-... by Southern (1975), who showed how ... molecules contained in an electrophore-... could be transferred to a membrane (UNIT ...) enabling genetic information relating to ...dual restriction fragments to be obtained ...ridization analysis.

...ce the pioneering work of Denhardt and ...rn, advances in membrane hybridiza-... have been technical rather than conceptual. ...ewed by Dyson (1991), the detailed ...ols have become more sophisticated, ... because of advances in understanding ...ctors that influence hybrid stability and ...zation rate.

...d stability is expressed as the melting ...ure or T_m, which is the temperature at ...e probe dissociates from the target ...r DNA-DNA hybrids, the T_m can be ...ated from the equation of Meinkoth ... (1984):

$$...5°C + 16.6(\log M) + 0.41(\%GC) - 0.61(\%form) - 500/L$$

...NA-DNA hybrids from the equation ...nd Davidson (1977):

$$...°C + 18.5(\log M) + 0.58(\%GC) - 11.8(\%GC)^2 - 0.56(\%form) - 820/L$$

...the molarity of monovalent cations, ... percentage of guanosine and cytosine nucleotides in the DNA, %form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The practical considerations that arise from these two equations are summarized Table 2.10.2A.

The second important consideration in hybridization analysis is the rate at which the hybrid is formed. Hybrid formation cannot occur until complementary regions of the two molecules become aligned, which occurs purely by chance; however, once a short nucleating region of the duplex has formed, the remaining sequences base-pair relatively rapidly. The rate at which the probe "finds" the target, which is influenced by a number of factors (Table 2.10.2B), is therefore the limiting step in hybrid formation (Britten and Davidson, 1985). However, in practical terms hybridization rate is less important than hybrid stability, as in most protocols hybridization is allowed to proceed for so long that factors influencing rate become immaterial.

Critical Parameters

To be successful, a hybridization experiment must meet two criteria:

(1) *Sensitivity.* Sufficient probe DNA must anneal to the target to produce a detectable signal after autoradiography.

(2) *Specificity.* After the last wash, the probe must be attached only to the desired target sequence (or, with heterologous probing, family of sequences).

Parameters influencing these two criteria are considered in turn, followed by other miscellaneous factors that affect hybridization.

Factors influencing sensitivity

The sensitivity of hybridization analysis is determined by how many labeled probe molecules attach to the target DNA. The greater the number of labeled probe molecules that anneal, the greater the intensity of the hybridization signal seen after autoradiography.

Probe specific activity. Of the various factors that influence sensitivity, the one that most frequently causes problems is the specific ac-

EXHIBIT A

e.g. expression vectors, the necessary common attribute is the ORF (SEQ ID NO: 2).

Weighing all factors including (1) that the full length ORF (SEQ ID NO: 2) is disclosed and (2) that any substantial variability within the genus arises due to addition of elements that are not part of the inventor's particular contribution, taken in view of the level of knowledge and skill in the art, one skilled in the art would recognize from the disclosure that the applicant was in possession of the genus of DNAs that comprise SEQ ID NO: 2.

Conclusion: The written description requirement is satisfied.

Example 9: Hybridization

Specification: The specification discloses a single cDNA (SEQ ID NO:1) which encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity. The specification includes an example wherein the complement of SEQ ID NO: 1 was used under highly stringent hybridization conditions (6XSSC and 65 degrees Celsius) for the isolation of nucleic acids that encode proteins that bind to dopamine receptor and stimulate adenylate cyclase activity. The hybridizing nucleic acids were not sequenced. They were expressed and several were shown to encode proteins that bind to a dopamine receptor and stimulate adenylate cyclase activity. These sequences may or may not be the same as SEQ ID NO: 1.

Claim:

An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1,

wherein said nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity.

Analysis:

A review of the full content of the specification indicates that the essential feature of the claimed invention is the isolated nucleic acid that hybridizes to SEQ ID NO: 1 under highly stringent conditions and encodes a protein with a specific function. The art indicates that hybridization techniques using a known DNA as a probe under highly stringent conditions were conventional in the art at the time of filing.

The claim is drawn to a genus of nucleic acids all of which must hybridize with SEQ ID NO: 1 and must encode a protein with a specific activity.

The search of the prior art indicates that SEQ ID NO: 1 is novel and unobvious.

There is a single species disclosed (a molecule consisting of SEQ ID NO: 1) that is within the scope of the claimed genus.

There is actual reduction to practice of the disclosed species.

Now turning to the genus analysis, a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of

skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

Conclusion: The claimed invention is adequately described.

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                                            10        20        30        40        50        60
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      ATG------AAGAAGTTTGTCG--CCCTGCTCATAACCATGTTTTTCGTAGTGAGCATGG
SEQ ID NO:125                       gtggtgcacatgaagtt-gaagtaccttgc-cttag---ttttgttggctgtg-gcttcg

                                            70        80        90       100       110       120
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      CT--GCCGTTGCACAGC--CAGCTAGCGCCGCAAAGTATTCCGAACTCGAAGAAGGCGGC
SEQ ID NO:125                       ataggcctactctcgactccagtgggtgctgccaagtactccgaactcgaagagggcggt

                                           130       140       150       160       170       180
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      GTTATAATGCAGGCCTTCTACTGGGACGTTCCAGCGGGAGGAATCTGGTGGGATACAATC
SEQ ID NO:125                       gttataatgcaggccttctactgggatgttcccggagggggaatctggtgggacaccata

                                           190       200       210       220       230       240
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      AGA-AGCAAGATACCGGAGTGGTACGAGGCTGGAATCTCCGCCATCTGGATTCCGCCAGC
SEQ ID NO:125                       agacagaaa-atcccggagtggtacgacgctggaatctcggcgatatggattcctccagc

                                           250       260       270       280       290       300
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      CAGCAAGGGCATGGGAGGAGCTTATTCAATGGGCTACGACCCATACGACTTCTTCGACCT
SEQ ID NO:125                       tagcaaagggatgggcggtggttattccatgggctacgatccctacgatttctttgacct

                                           310       320       330       340       350       360
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      CGGCGAGTACAACCAGAAGGGAACAGTTGAAACTCGCTTTGGCTCAAAGCAGGAGCTTAT
SEQ ID NO:125                       cggcgagtactatcagaagggaacagttgagacgcgcttcggctcaaaggaggaactggt

                                           370       380       390       400       410       420
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      CAACATGATAAACACCGCCCATGCCTACGGCATAAAGGTCATAGCTGATATCGTCATAAA
SEQ ID NO:125                       gaacatgataaacaccgcacactcctatggcataaaggtgatagcggacatagtcataaa

                                           430       440       450       460       470       480
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      CCACCGCGCGGGCGGAGACCTCGAGTGGAACCCGTTCGTTGGGGACTACACCTGGACGGA
SEQ ID NO:125                       ccaccgcgccggtggagaccttgagtggaacccctttgtaaacaactatacttggacaga

                                           490       500       510       520       530       540
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      CTTCTCGAAGGTGGCCTCGGGCAAATATACCGCCAACTACCTCGACTTCCACCCCAACGA
SEQ ID NO:125                       cttctccaaggtcgcctccggtaaatacacggccaactaccttgacttccacccaaacga

                                           550       560       570       580       590       600
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      GGTCAAGTGCTGTGACGAGGGCACATTTGGAGGTTTCCCAGACATAGCCCACGAGAAGGA
SEQ ID NO:125                       ggtcaagtgctgcgatgagggtacatttggtgactttccggacatcgcccacgagaag-a

                                           610       620       630       640       650       660
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      G-TGGGACCAGCACTGGCTCTGGGCGAGCGACGAGAGCTACGCCGCCTACCTCAGGAGCA
SEQ ID NO:125                       gctgggatcagtactggctctgggcaagcaatgagagctacgccgcatatctccggagca

                                           670       680       690       700       710       720
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      TCGGCGTTGATGCCTGGCGTTTCGACTACGTAAAGGGCTACGGAGCGTGGGTCGTCAAGG
SEQ ID NO:125                       tagggatcgatgcatggcgtttcgactacgtcaaaggttacggagcgtgggttgttaatg

                                           730       740       750       760       770       780
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      ACTGGCTCAACTGGTGGGGCGGCTGGGCCGTCGGTGAGTACTGGGACACGAACGTTGATG
SEQ ID NO:125                       actggctcagctggtggggaggctgggccgttggagagtactgggacacgaacgttgatg

                                           790       800       810       820       830       840
                                    ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus      CACTCCTCAATTGGGCATACTCGAGCGGCGCCAAGGTCTTCGACTTCCCGCTCTACTACA
SEQ ID NO:125                       cactccttaactgggcatacgacagcggtgccaaggtctttgacttcccgctctactaca
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EXHIBIT C

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                                     850       860       870       880       890       900
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus   AAATGGACGAGGCCTTTGACAACACCAACATCCCGGCCTTGGTTGATGCCCTCCAGAACG
SEQ ID NO:125                    agatggacgaagcctttgacaacaccaacatccccgctttggtttacgccctccagaacg

                                     910       920       930       940       950       960
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus   GGGGAACCGTCGTCTCTCGCGACCCGTTCAAGGCCGTAACCTTTGTAGCAAACCACGACA
SEQ ID NO:125                    gaggaacagtcgtttcccgcgatcccttcaaggcagtaactttcgttgccaaccacgata

                                     970       980       990      1000      1010      1020
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus   CCGATATAATCTGGAACAAGTACCCTGCTTATGCTTTCATCCTCACCTACGAGGGCCAGC
SEQ ID NO:125                    cagatataatctggaacaagtatccggcttatgcgttcatcctttacctatgagggacagc

                                    1030      1040      1050      1060      1070      1080
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus   CCGTCATATTCTACCGCGACTACGAGGAGTGGCTCAACAAGGACAAGCTTAACAACCTAA
SEQ ID NO:125                    ctgttatatttaccgcgactacgaggagtggctcaacaaggataagcttaacaacctta

                                    1090      1100      1110      1120      1130      1140
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus   TCTGGATACACGACCACCTCGCGGGTGGAAGCACGAGCATAGTCTACTACGACAGCGACG
SEQ ID NO:125                    tctggatacacgagcaccttgccggaggaagtaccaagatcctctactacgataacgatg

                                    1150      1160      1170      1180      1190      1200
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus   AGCTGATCTTCGTGAGGAACGGCGACTCCAAGAGGCCGGGACTGATAACGTACATCAACC
SEQ ID NO:125                    agctaatattcatgagggagggctacgggagcaagccgggcctcataacctacataaacc

                                    1210      1220      1230      1240      1250      1260
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus   TCGGCT-CTAGCAAGGTCGGAAGGTGGGTGTACGT--GC-CGAAGTTCGCGGGCGCGTGC
SEQ ID NO:125                    tcggaaacga-ctgggccgagcgctgggtgaacgtcggctcaaagtttgccggctacaca

                                    1270      1280      1290      1300      1310      1320
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus   ATCCACGAGTACACCGGCAACCTCGGAGGCTGGGTAGACAAGTACGTCGAGT-CGAGCGG
SEQ ID NO:125                    atccatgaatacacaggcaatctcggtggctgggttgacaggtgggttcagtacgat-gg

                                    1330      1340      1350      1360      1370      1380
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
gi|2251107:452-1837 Pyrococcus   CTGGGTCTATCTCGAAG-CTCCAGCTTACGACCCCGCCAGCGGGCAGTACGGCTACACCG
SEQ ID NO:125                    atgggttaaact-gacggcacctcctcatgatccagccaacggatattacggctactcag

                                    1390      1400
                                 ....|....|....|....|....|..
gi|2251107:452-1837 Pyrococcus   TCTGGAGCTACTGCGGC-GTTGGATGA
SEQ ID NO:125                    tctggagctac-gcaggcgtcggatga
```

```
                                            10        20        30        40        50        60
                                   ....|....|....|....|....|....|....|....|....|....|....|....|
SEQ ID NO:126                      ----VVHMKLKYLALVLL----------AVASIGLL---STPVGAAKYSELEEGGVIMQA
gi|2251108|dbj|BAA21130.1| alp     MKKF-V-------A--LLITMFFVVSMAAVA-----QPAS----AAKYSELEEGGVIMQA

                                            70        80        90       100       110       120
                                   ....|....|....|....|....|....|....|....|....|....|....|....|
SEQ ID NO:126                      FYWDVP-GGGIWWDTIRQ-KIPEWYD-AGISAIWIPPASKGMGGG-YSMGYDPYDFFDLG
gi|2251108|dbj|BAA21130.1| alp     FYWDVPAGG-IWWDTIR-SKIPEWY-EAGISAIWIPPASKGMGG-AYSMGYDPYDFFDLG

                                           130       140       150       160       170       180
                                   ....|....|....|....|....|....|....|....|....|....|....|....|
SEQ ID NO:126                      EYY-QKGTVETRFGSK-EELV-NMINTAHS-YGIKVIADIVINHRAGGDLEWNPFVNN--
gi|2251108|dbj|BAA21130.1| alp     EY-NQKGTVETRFGSKQE-L-INMINTAH-AYGIKVIADIVINHRAGGDLEWNPFV--GD

                                           190       200       210       220       230       240
                                   ....|....|....|....|....|....|....|....|....|....|....|....|
SEQ ID NO:126                      YTWTDFSKVASGKYTANYLDFHPNEVKCCDEGTFGD-FPDIAHEKS-WDQY-WLWASN-E
gi|2251108|dbj|BAA21130.1| alp     YTWTDFSKVASGKYTANYLDFHPNEVKCCDEGTFG-GFPDIAHEK-EWDQ-HWLWAS-DE

                                           250       260       270       280       290       300
                                   ....|....|....|....|....|....|....|....|....|....|....|....|
SEQ ID NO:126                      SYAAYLRSIGI-DAWRFDYVKGYGAWVVN-DWL-SWWGGWAVGEYWDTNVDALLNWAYDS
gi|2251108|dbj|BAA21130.1| alp     SYAAYLRSIG-VDAWRFDYVKGYGAWVV-KDWLN-WWGGWAVGEYWDTNVDALLNWAY-S

                                           310       320       330       340       350       360
                                   ....|....|....|....|....|....|....|....|....|....|....|....|
SEQ ID NO:126                      -GAKVFDFPLYYKMDEAFDNTNIPALVY-ALQNGGTVVSRDPFKAVTFVANHDTDIIWNK
gi|2251108|dbj|BAA21130.1| alp     SGAKVFDFPLYYKMDEAFDNTNIPALV-DALQNGGTVVSRDPFKAVTFVANHDTDIIWNK

                                           370       380       390       400       410       420
                                   ....|....|....|....|....|....|....|....|....|....|....|....|
SEQ ID NO:126                      YPAYAFILTYEGQPVIFYRDYEEWLNKDKLNNLIWIHE-HLAGGSTKIL---YYDN-DEL
gi|2251108|dbj|BAA21130.1| alp     YPAYAFILTYEGQPVIFYRDYEEWLNKDKLNNLIWIH-DHLAGGST---SIVYYD-SDEL

                                           430       440       450       460       470       480
                                   ....|....|....|....|....|....|....|....|....|....|....|....|
SEQ ID NO:126                      IFMREGY---G-SK-PGLITYINLGNDWAE-----RWVNVGS---KFAGYT--IHEYTGN
gi|2251108|dbj|BAA21130.1| alp     IF-----VRNGDSKRPGLITYINLG-----SSKVGRWV----YVPKFAG--ACIHEYTGN

                                           490       500       510       520       530
                                   ....|....|....|....|....|....|....|....|....|....|....|..
SEQ ID NO:126                      LGGWVDRWVQYD------GWVK-LTAP--PH--DPANGY---YGYS-VWSYA-GVG*
gi|2251108|dbj|BAA21130.1| alp     LGGWVD------KYVESSGWV-YL---EAP-AYDPA---SGQYGY-TVWSY-CGVG-
```

EXHIBIT D

Atty Docket No.: 56446-20061.00 /09010-108001/DIVER1530-5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Walter Callen et al. Art Unit : 1652
Serial No. : 10/081,872 Examiner : Rebecca E. Prouty, Ph.D.
Filed : February 21, 2002
Title: Enzymes Having Alpha Amylase Activity and Methods of Use Thereof

MS RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay Short, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as CEO and as research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume was provided with my previous declaration submitted in connection with this application.

2. It was considered routine by one skilled in the art to make multiple substitutions or multiple modifications in a nucleic acid sequence, such as a polypeptide coding sequence, and screen for variant enzymes, such as amylases, encoded by the modified nucleic acids. The state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art for screening enzymes for amylase activity was very high. It would not have been necessary for the skilled artisan to understand which regions of the amylases encoded by nucleic acids used in the claimed methods could be modified to gain a function or activity, or, modified without loss of a function or activity, for example, amylase activity. It would not have been necessary for the skilled artisan to understand which specific regions or structural elements of the exemplary sequences were needed to have amylase function or activity (e.g., amylase activity) to routinely generate the genus of amylase-encoding nucleic acids used in the claimed methods. Methods for making and screening sequence modifications and enzyme fragments were sufficiently comprehensive, routine and predictable at the time of the invention to predictably generate a genus of amylase-encoding sequences without need of knowing which

specific regions or structural elements of a sequence or structure affected function or activity (e.g., amylase activity). Methods known at the time of the invention for modifying nucleic acid and polypeptide sequences in combination with high through-put enzyme (amylase) screening made methods that required previous knowledge of structural elements necessary for enzymatic activity obsolete and unnecessary. Methods known at the time of the invention for modifying nucleic acid and polypeptide sequences in combination with high through-put enzyme (amylase) screening known at the time of the invention, made methods that require previous knowledge of protein structure, including secondary or tertiary structure, active site sequences, and the like obsolete and unnecessary. At the time of the invention, high through-put *in vivo* (e.g., whole cell) nucleic acid expression and enzyme (amylase) screening protocols were well known in the art. The specification sets forth exemplary amylase screening assays to determine if a polypeptide is within the scope of the genus used in the claimed methods (see, e.g., Examples 1-2 and 5-10, of the specification). Using methods known in the art at the time of the invention it would not have been necessary to understand which specific regions of amylase structure needed to be modified to generate a genus of nucleic acids or polypeptides for practicing the claimed methods without undue experimentation. The specification presented to the skilled artisan a rational and predictable scheme for making the genus of amylases and amylase-encoding sequences, including a rational and predictable scheme for modifying the exemplary SEQ ID NO:125 with an expectation of obtaining an enzymatically active genus of amylase-encoding nucleic acids or amylase enzymes, including amylases. The specification provided sufficient guidance to one of ordinary skill in the art to make and use the genus of amylase-encoding nucleic acids to practice the methods of the invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date:________________________ ________________________________

Jay Short

CURRICULUM VITAE

NAME: Jay M. Short, Ph.D.

EDUCATION:

1981 - 1985	Ph.D., Biochemistry Case Western Reserve University, Cleveland, Ohio
1980 - 1981	Graduate Study, Macromolecular Science Case Western Reserve University, Cleveland, Ohio
1976 - 1980	B.A. with Honors, Chemistry Taylor University, Upland, Indiana

RESEARCH & PROFESSIONAL EXPERIENCE:

1999 - present	**CEO, President, Chief Technology Officer & Board of Directors** Diversa Corporation San Diego, California
1998 - present	**President, Chief Technology Officer & Board of Directors** Diversa Corporation San Diego, California
1997 - 1998	**Executive Vice President, Chief Technology Officer & Board of Directors** Diversa Corporation San Diego, California
1994 - 1997	**Chief Technology Officer & Board of Directors** Diversa Corporation San Diego, California
1990 - 1994	**President** Stratacyte, Inc. La Jolla, California
1992 - 1994	**Vice President** R&D (Research) and Operations Stratagene Cloning Systems La Jolla, California
1989 - 1992	**Vice President** R&D (Research) and Biological Operations Stratagene Cloning Systems
1988 - 1989	**Senior Staff Scientist** Research and Development Stratagene Cloning Systems

1985 - 1988 **Staff Scientist**
Research and Development
Stratagene Cloning Systems

1981 - 1985 **Ph.D. Research**
Case Western Reserve University
Dr. Richard W. Hanson's Laboratory,
Identification and characterization of the promoter for
P-enolpyruvate carboxykinase.
First identification of a cAMP regulatory domain.

1980 - 1981 **Graduate Student Research**
Case Western Reserve University
Dr. Bruce Roe's Laboratory, Analysis of the cellulase activity of *Trichoderma viride*.

TEACHING EXPERIENCE:

Thesis Advisor (1988-1993), University of Uppsala, Sweden, Ph.D. for Michelle Alting-Mees
Lecturer (1992), Committee for Advanced Scientific Education, Center for Drug Evaluation and Research, FDA.
Faculty (1989), Transgenic Mouse Model and its Application in Assessing *In Vivo* Mutagenesis, Genetic Toxicology Workshop (3rd Annual). Microbiological Associates Inc. Bethesda, MD.
Faculty (1987), DNA Cloning and Expression. Physiology Society Workshop. San Diego, CA.
Teaching Assist., (1981-1985). Molecular and Cellular Biology. Case Western Reserve University.
Teaching Assist., (1981). Physiological Chemistry. Kent State Univ., Kent, OH.
Teaching Assist., (1978-1980). Quantitative Analysis. Taylor University.

AWARDS, PROFESSIONAL MEMBERSHIPS, ACCOMPLISHMENTS, AND ACTIVITIES:

Visiting Scientist, International Centre of Insect Physiology and Ecology (ICIPE), Kenya (2002-2004)
Science & Technology Committee, *BIOCOM San Diego*
Advisory Board, IngleWood Ventures
Finalists for UCSD Connect's Most Innovative New Product Award in the Biotechnology R&D Category
Advisory Board, *Chemical & Engineering News*
Board of Advisors and Founding Member, Division of Biological Sciences, *UCSD*
Board Director, *BIOCOM San Diego*
Chairman of the Board, Innovase
Board Director, Zymetrics
Board Director, Innovase
Director at Large, *YPO (Young Presidents' Organization) San Diego.*
2001 T-Sector Life Science Innovator Award.
2001 Deloitte and Touche's Orange County / San Diego 2001 Technology "Fast 50".
San Diego Entrepreneur of the Year 2001.
YPO (Young Presidents' Organization) San Diego.
YPO (Young Presidents' Organization) International.
Finalist for San Diego Entrepreneur of the Year in 2000.
Largest Biotechnology IPO raising over $200MM.
Founding management member of Diversa Corporation.
Panel for Chemical Science & Technology for NIST, appointed by the National Research Council (1997-2000).
Chairman (1993), Discussion Group, Society of Toxicology Conference.
U.S. Committee Member for Evaluation of Biotechnology Research in Spain.
Editor, Mutation Research.

UCSD Connect Program (1991) 1st Place Award for Innovation and Entrepreneurship in Biotechnology (over 50 competing biotech companies).
UCSD Connect Program (1990) 1st Place Award for Innovation and Entrepreneurship in Biotechnology.
Consultant for European Economic Community on Transgenic Toxicology Testing (91-94).
The New York Academy of Sciences.
Reviewer for *Proceedings of the National Academy of Sciences, Genetic Analysis Techniques, Analytical Biochemistry, & Nucleic Acids Research.*
American Association for the Advancement of Science.
American Chemical Society.
American Society of Biochemistry and Molecular Biology.
American Society of Microbiology.
Environmental Mutagenesis Society.
Society for Industrial Microbiology
Society of Toxicology.
Japanese Environmental Mutagen Society.
Who's Who Registry of Business Leaders (1994-1995)
American Men and Women of Science (1995)
NIEHS Peer Review Committee.
SBIR Study Section.
SBIR Annual Report (1993) Program Success Profile (Top 8 of 800 Companies).
Stratagene (1990) Innovation Award - Lambda ZAP® vector.
Stratagene (1990) Service Award
Stratagene (1991) Innovation Award - Big Blue® Transgenic Testing System.
Stratagene (1992) Most Innovative Award - Managers/Supervisors.
Institutional Animal Care and Use Committee (IACUC), Chairman and Institutional Official.
Award from the University of Victoria for Contributions to the Development of Short-term Transgenic Mutation Assays.
Nominated as Council Member for the U.S. Environmental Mutagen Society.
Board Director, *Stressgen (TSE), Victoria, BC, Canada*
Board Director & Treasurer, *Stressgen Therapeutics, Victoria, BC, Canada*
Board Director & Secretary, *Stressgen Therapeutics, Victoria, BC, Canada*
Board Director, *Diversa, La Jolla, CA*
Board Director, *Invitrogen, Carlsbad, CA*
Consultant, *Stratagene Cloning Systems, La Jolla, CA*
Consultant, *Micro Product Systems, Lynn, IN*
Reviewer for U.S. Congressional Office of Technology Assessment (OTA) on *The Human Genome Project and Patenting DNA Sequences.*

MEDIA:

ABC Discovery News, ABC San Diego Channel 10, BBC Radio, Bioinformed Newsletter, Biotechnology Newsletter, BioVentures View, Business Daily, Business Week, CEO Cast, Chemical Engineering, Chemical Week, Chemistry & Industry (UK), CNBC, CNN Science & Technology, dBusiness.com, Discovery Magazine, Forbes.com, Good Morning America, Horizon Air Magazine, Idea TV, Inside Business Radio Show, JAG Financial News, Los Angeles Times, NBC San Diego Channel 7/39, National Radio Report, New York Times, Pirateinvestor.com, R&D Magazine, RTL German Television, Reuters, San Diego Business Transcript, San Diego Channel KUSI, San Diego Channel 10, San Diego Magazine, San Diego Union Tribune, Scientist, Time Magazine, Stewards' Watch, The Discovery Channel, The Motley Fool, Time Magazine, USA Today, Wall Street Journal, Wall Street Transcript, Washington Post

PATENTS:

DNA Cloning Vectors with *in vivo* Excisable Plasmids (1987).
Mutagenesis Testing Using Transgenic Animals Carrying Marker Genes (1987).
Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test

DNA Sequences (1987).
Dietary and Hormonal Regulation of Expression of Exogenous Genes in Transgenic Animals Under Control of the Promoter of the Gene Phosphoenolpyruvate Carboxykinase (1988).
A Transgenic Mouse for Measurement and Characterization of Mutation Induction *In Vivo* (1989).
Rapid Screening Mutagenesis and Teratogenesis Assay (1989).
A Combinatorial Approach to Regenerating the Immunoglobulin Repertoire in Prokaryotic Cells (1990).
Transgenic Animal Models for *In Vivo* Mutagenesis Testing (1990).
Polycos Vectors (1991).
A Lambda Packaging Extract Lacking β-Galactosidase Activity (1991).
A System for Regulation of Eukaryotic Genes (1991).
Methods for Phenotype Creation from Multiple Gene Populations (1991).
Transgenic Non-Human Animals Carrying Test DNA Sequences (1992).
Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test DNA Sequences (1992).
Selectable System Patent (1992).
Polycos Mutagenesis Systems (1993).
Use of Trans-acting Proteins for the Development of an *In Situ* Expression Screening System (1993).
Enzyme Kits and Libraries (1995).
Enzyme Activity Screening of Clones having DNA from Uncultivated Microorganisms (1995).
Enzyme Tiered (1995).
Method for Screening for Enzyme Activity (1995).
Combined Enzyme Screening/Evolution (1995).
Uncultured/Activity Screening (1995).
Directed Evolution of Thermophilic Proteins (1995).
Combinatorial Enzyme Development (Directed Mutagenesis) (1996).
Protein Activity Screening of Clones having DNA from Uncultivated Microorganisms (1996).
Production and Use of Normalized DNA Libraries (1996).
Methods of DNA Shuffling with Polynucleotides Produced by Blocking or Interrupting a Synthesis or Amplification Process (1996).
Method of Screening for Enzyme Activity (Biopanning) (1996).
Directed Evolution of Thermophilic Enzymes (1996).
Environmental Biopanning (1996).
Combinatorial Enzyme Development (1996).
Enzyme Activity Screening of Clones Having DNA from Uncultivated Miroorganisms (1996).
Normalized Samples/Libraries (1996).
Reassembled Pools of Mutagenized DNA & Procedure (1996).
Fluorescent-based Single Screening for Enzymes (1996).
High Throughput Screening for Novel Enzymes (1997).
Nucleotide Sequence of the *Aquifex aeolicus* Genome, Fragments Thereof, and Uses Thereof (1997).
Screening for Novel Bioactivities (1997).
Screening for Novel Compounds which Regulate Biological Interactions (1997).
Method for Screening Enzyme Activity (1997).
High Throughput Screening for Novel Enzymes (1997).
"Discovery" (whole process, including uncultivated, normalized, biopanning, screening, evolving, (etc.) (1997).
Production of Enzymes Having Desired Activities By Mutagenesis (1999).
Protein Activity Screening of Clones Having DNA from Uncultivated Microorganisms (1999).
Method of DNA Reassembly by Interrupting Sythesis (1999).
Production and Use of Normalized DNA Libraries (1999).
Enzyme Kits and Libraries (1999).
Screening for Novel Bioactivities (2000).
Method for Screening for Enzyme Activity (2000).
Screening for Novel Bioactivities (2000).
Production and Use of Normalized DNA Libraries (2000).
Method of Screening for Enzyme Activity (2000).
Screening Methods for Enzymes and Enzyme Kits (2001).
Saturation Mutagenesis in Directed Evolution (2001).
High Throughput Screening for Novel Enzymes (2001).

Recombinant Bacterial Phytases and Uses Thereof (2001).
Methods Useful for Nucleic Acid Sequencing Using Modified Nucleotides Comprising Phenylboronic Acid (2001).
End Selection in Directed Evolution (2001)
Gene Expression Library Produced From DNA From Uncultivated Microorganisms and Method for Making the Same (2001)
Directed Evolution of Thermophilic Enzymes (2002)
Method for Screening for Enzyme Activity (2002)
Exonuclease-Mediated Gene Assembly in Directed Evolution (2002)
End Selection In Directed Evolution (2002)
Exonuclease-Mediated Gene Assembly in Directed Evolution (2002)
Screening for Novel Bioactivities(2002)
Method of DNA Shuffling with Polynucleotides Produced or Blocking or Interrupting Synthesis or Amplification Process (2002)
Production and Use of Normalized DNA Libraries (2002)
Sequence Based Screening (2002)
Non-Stochastic Generation of Genetic Vaccines (2002)
Over 100 Additional Pending Patent Applications Worldwide.

GRANTS AND CONTRACTS:

*Phase I Small Business Contract #N43-Am-62282. 1985 - 1986. P.I.
Vectors and Techniques for Rapid DNA Sequencing.
*Phase II Small Business Contract #N43-Am-62282. 1988 - 1990. P.I.
Vectors and Techniques for Rapid DNA Sequencing.
*Phase I Small Business Grant 2R43ES04484-02. 1986 - 1987. P.I.
Identification of Genetic Lesions Leading to Mutations.
*Phase II Small Business Grant 2R43ES04484-02. 1989 - 1992. P.I.
Identification of Genetic Lesions Leading to Mutations.
*1R01-ES04728-01A1. 1989 - 1992. (NIEHS) P.I.
Animal Model for Identification of Genetic Lesions.
*Phase I Small Business Grant #R43GM42291-01. 1989. P.I.
Switch Mechanism for Gene Expression in Transgenics.
*RFP NIH-ES-88-11. 1989-1994. (NIEHS) Co-I.
Development of Mutagenesis Assays Using Transgenic Mice.
*Phase II Small Business Grant #2R44GM42291-02. 1990-1992. (DRG/NIH) P.I.
Switch Mechanism for Gene Expression In Transgenics.
*Phase I Small Business Grant #1R43GM46585-01. 1991. (DRG/NIH) P.I.
Generation of a Peptide Screening System Through the Development of Combinatorial-splicing "Polycos" Vectors.
*Phase I Small Business Grant #1R43CA57066-01. 1992. (NCI) P.I.
Transgenic Rats: A Short-term Mutagenicity Assay for Multi-species Testing of Suspected Human Carcinogens.
*Phase I Small Business Grant #1R43GM48300-01. 1992. (DRG/NIH) P.I.
Analysis of the Immunoglobulin Hypermutator Mechanism.
*Phase I Small Business Grant #1R43ES06146-01. 1992. (NIEHS) P.I.
Selectable "Polycos" Shuttle Vectors for In Vivo Mutagenicity Testing.
*Phase II Small Business Grant #2R44GM46585-02. 1992-1994. (NIGMS) P.I.
Peptide Screening Utilizing Combinatorial Polycos Vector.
*Phase I Small Business Grant #1R43RR08667-01. 1992-1993. (DRG/NIH) Co-I.
A One-step PCR Cloning System Based on FLP Recombination.
*Phase II Small Business Grant #2R44CA57066-02. 1993-1995. (NCI) P.I.
Transgenic Rats:Transgenic Rat Model for Mutagenicity Testing.
*Phase I Small Business Grant. 1993-1994. (NIH) Co-I.
Transgenic Fish Model for Mutagenicity Testing.
*Phase II Small Business Grant (1994-1996). (NIH) P.I.
"Polycos" Shuttle Vectors for Mutagenicity testing.
*Phase I Small Business Grant. 1994. (NIH) Co-I.
Vector System for Studying Protein-Protein Interactions.

*CRADA with LLNL. 1994. (NIH) Co-I.
Mouse and Rat Painting Probes.
*CRADA with FDA. 1994. (NIH) Co-I.
Tamoxifen Testing in F-344 Rats.
*CRADA with NASA. 1994. (NIH) Co-I.
Radiation Damage in the Microgravity Environment.

ABSTRACTS AND INVITED LECTURES:

Over 200 Abstracts and Invited Lectures.

PUBLICATIONS:

1. Yoo-Warren, H., Monahan, J.E., Short, J.M., Short, H., Bruzel, A., Wynshaw-Boris, A., Meisner, H.M., Samols, D., and Hanson, R.W. (1983) Isolation and Characterization of the Gene Coding for Cytosolic Phosphoenolpyruvate Carboxykinase (GTP) from the Rat. *Proc. Natl. Acad. Sci. U.S.A.*, 80:3656-3660.

2. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1984) Identification of cAMP Regulatory Region in the Gene for Rat Cytosolic Phosphoenolpyruvate Carboxykinase (GTP): Use of Chimeric Genes Transfected into Hepatoma Cells. *J. Biol. Chem.*, 259:12161-12169.

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9. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1987) *cis* - acting Regulatory Elements in Hormonally Responsive Genes. In: *Progress in Nucleic Acid Research and Molecular Biology* (W.E. Cohn and K. Moldave eds.), Academic Press, Inc., Orlando, Florida, 34:59-87.

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20. Alting-Mees, M., Amberg, J., Ardourel, D., Elgin, E., Greener, A., Gross, E.A., Kubitz, M., Mullinax, R.L., Short, J.M., and Sorge, J.A. (1990) Monoclonal Antibody Expression Libraries:A Rapid Alternative to Hybridomas. *Strategies in Mol. Biol.*, 3:1-9.

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23. Kretz, P., Kohler, S., and Short, J.M. (1990) The Effect of *E. coli* Minute 98 Locus on DNA Containing Eukaryotic Modifications. *Strategies In Mol. Biol.*, 3:21-22.

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